Conductine Protein and a Related Agent For Diagnosing and Treating Tumor Illnesses

The invention relates to a new way of combating tumor diseases by utilizing molecular biological relationships of the formation of tumors. In particular, it relates to a material for diagnosing tumor diseases and, based on this, a material for the treatment. It furthermore relates to the new protein, conductine, its mutants and variations as well as to parts thereof, to the analogous cDNA sequences and to their use in the gene-therapeutic and pharmacological methods. Areas of the application are medicine and the pharmaceutical industry.

Cadherines and catenines form cell adhesion complexes, which are responsible in numerous tissues for the adhesion of cells to one another. The cadherines are trans-membrane proteins and produce the direct contact between adjacent cells. a-, b- and g-catenine are cytoplasmic components, which connect the cadherines with the actin cytoskeleton. Aside from their function in cell adhesion, the catenines also play a decisive role in signal transduction processes. b-Catenine in vertebrates and the homologous, segment polarity gene product, armadillo in drosophila, are stabilized by the Wnt/wingless signal path (Nusse, R., Cell 89, 321 – 323, 1997). This leads to an increase in the cytoplasmic fraction of these proteins, which is not bound to cadherine, which thereupon could interact with HMG transcription factors of the LEF-1/TCF families. As a result, b-catenine/armadillo is transported into the cell nucleus where, together with the LEF/TCF proteins, it binds to the DNA and activates certain genes (Behrens, J. et al., Nature 382, 638 – 642, 1996).

This signal path also plays an important role in the formation of tumors. In epithelial cells of the colon, the cytoplasmic pool of b-catenine is strictly regulated by the tumor suppressor gene product APC (Adenomatosis Polyposis Coli). Mutations of APC, such as those occurring in 80% of all colon cancers, lead to shortened forms of the APC protein, which are no longer able to destabilize b-catenine. As a result, permanent complexes of b-catenine with the HMG transcription factor TCF-4, which are made responsible for the transformation of the cells, are found in these tumors. This theory is supported by the recent finding that, in tumors in which APC is not changed, mutations of b-catenine occur. These also lead to cytoplasmic stabilization of b-catenine and to an association with LEF-1/TCF factors (Morin, P.J. et al., Science 275, 1787 – 1790).

The invention has the goal of finding a new way for preventing the formation of tumors. It is based on the objective of finding a method for controlling the regulation of b-catenine in cells of the body.

The object of the invention is a new protein, which binds to b-catenine and leads to its cytoplasmic breakdown. This protein has the amino acid sequence of Figure 1 and was given the name of conductine.

The invention is based on our own realization that conductine binds to APC fragments over a b-catenine binding domain at b-catenine, over a GSK 3b-binding domain at GSK 3b and over a so-called RGS domain (regulator of G-protein signaling). As a result, there is cytoplasmic degradation of b-catenine and in vertebrates, blockage of the Wnt/wingless signal path. With that, it is clear that conductine is an important regulator of the b-catenine function and, in interaction with APC, contributes to the suppression of tumors.

Furthermore, as a consequence, the invention relates to a material for diagnosing tumor diseases, which is characterized in that the presence and the amount of conductine, its mutants and variations or its parts is detected in cells of the body. This detection can be carried out on the protein level with specific antibodies, especially with monoclonal antibodies.

Pursuant to the invention, the diagnosis of tumor diseases can also be carried out on the gene level. For this purpose,

- the gene, which codes for conductine, its mutants and variations or parts thereof and/or
- mRNA sequences, which are read by these genes,

are detected with selected primers and cDNA probes, which are derived from the gene sequence of the conductine.

The inventive material for the treatment of tumor diseases contains substances which activates/reactivate the action of the conductine in the body. Above all, these are materials, which activate the gene promoter of conductine or materials, which increase the stability of the mRNA sequences derived from the conductine genes. Pursuant to the invention, the main objective of all of these materials consists of increasing the activity of the conductine in the cells of the body. For this purpose, substances of low molecular weight, for example, come into consideration, which are found, for example, by high throughput number screening.

The invention also comprises gene therapeutic materials, containing genes, which code for conductine, its mutants and variations or parts thereof, or mRNA sequences, which are read by these genes.

Furthermore, the new proteins conductine of Figure 1 - SEQ ID No. 1 its mutants and variations, as well as parts thereof are placed under protection. Especially preferred partial sequences are the amino acids 78 to 200 (RGS) – SEQ ID No. 2, 343 - 396 (GSK 3b-binding domains) – SEQ ID. No. 3, 397 - 465 (b-catenine binding domains) – SEQ ID No. 4 and 783 - 833 (disheveled homology region) – SEQ ID No. 5. Partial sequences of the Adenomatosis Poliposis Coli (APC), which are characterized by the amino acid sequences 1464 – 1604, 1516 – 1595, 1690 – 1778 and 1995 – 2083 as RGS-domains interaction sites, are also part of the extent of the protection.

Likewise, the analogous cDNA sequences, especially the full cDNA sequence of the conductine (base pairs 1 – 2825) of Figure 2 – SEQ ID No. 6, as well as the partial sequences of the conductine of the nucleotide sequence 446 – 814 (RGS gene section) – SEQ ID No. 7, of the nucleotide sequence 1241 – 1402 (gene section of GSK 3b-binding domains) – SEQ ID No. 8, 1403 – 1609 (gene section of the b-catenine binding domains) – SEQ ID No. 9 and of the nucleotide sequence 2561 – 2713 (gene section of the disheveled homology region) – SEQ ID No. 10.

The invention is explained in greater detail by the following examples.

Conductine was identified by a yeast 2-hybrid screen as a b-catenine interaction partner. The complete cDNA sequence was subsequently isolated and sequenced. The derived amino acid sequence of conductine is shown in Figure 1, the nucleotide sequence in Figure 2 and the gene comparison of the amino acid sequence and the nucleotide sequence is shown in Figure 3. Conductine consists of 840 amino acids and has a molecular weight of 92.8 kDa. By a comparison of sequences, an RGS domain (amino acid 78 – 200) and a domain (amino acid 783 –

833, disheveled homology region), related to the protein disheveled, were identified (Figures 1 – 3). The GSK 3b- and b-catenine binding domains (amino acids 343 – 396 to 397 – 465) were discovered by interaction studies in the 2-hybrid system (Figure 4). It was observed that these domains are sufficient and necessary for the binding to GSK 3b or to b-catenine (Figure 4). On the other hand, the RGS homology region and the disheveled homology region do not participate. The interaction of conductine with GSK 3b and b-catenine was also confirmed biochemically in co-immunoprecipitation experiments.

The effect of conductine on b-catenine was investigated in SW480 cells. In these cells, the tumor suppressor gene product APC is mutated, as a result of which there is an increase in the cytoplasmic and especially in the nuclear content of b-catenine. The introduction of conductine into these cells leads to a drastic breakdown of b-catenine, as a result of which the cells are depleted of cytoplasmic b-catenine and of b-catenine in the cell nucleus (Figure 4). This effect on the content of b-catenine is equal in intensity to that of not-mutated APC, from which it can be concluded that conductine also acts as a tumor suppressor by regulating b-catenine. Moreover, it was shown that conductine also inhibits the Wnt/wingless signal path in Xenopus embryos due to its effect on b-catenine.

Furthermore, it was noted that conductine interacts directly with APC. APC fragments of amino acids 1464 – 1604, 1516 – 1595, 1690 – 1778 and 1995 – 2083 were identified as interaction sites for conductine. In conductine, the binding to APC takes place over the RGS domains; this region is sufficient and necessary for the interaction. The other domains in conductine do not participate (Figure 4).

Legends for the Figures

Figure 1

Amino Acid Sequence of Conductine

The conductine cDNA codes a protein of 840 amino acids with a calculated molecular weight of 92.8 kDa. The RGS domains (double underlining), the b-catenine binding domains (simple underlining) and the disheveled homology region are emphasized by bold lettering.

Figure 2

Nucleotide Sequence of Conductine at Position 1 – 2825

The sequence regions are marked as in Figure 1.

Figure 3

Comparison of Amino Acid Sequence and Nucleotide Sequence of Conductine

Figure 4

Analysis of the Interaction of Conductine and its Parts with b-Catenine, APC and GSK 3b

The conductine protein and derived partial pieces are shown diagrammatically. The RGS domains (RGS), the GSK 3b-binding domains (GSK BD) and the b-catenine binding sites (b-BD) are emphasized. The interaction with b-catenine with the APC fragments of amino acids 1464 – 1604 (APCfr.1) and 1516 – 1595 (APCfr. 2) and GSK 3b were investigated in the yeast 2-hybrid assay and quantified as b-galactosidase units. It can be seen that the binding of the b-catenine to the b-

catenine binding site is limited; the other parts of the protein do not contribute to this. The analysis furthermore shows the exclusive interaction of APC with the RGS domains of conductine. Comparable results for the binding to the RGS domains were obtained with the APC fragments of amino acids 1690 – 1778 and 1995 – 2083. The breakdown of b-catenine into SW480 cells by conductine was analyzed after transient expression of the given proteins and immunofluorescence staining of b-catenine. Only partial pieces of conductine, which bind to b-catenine, lead to this breakdown. The analysis finally shows the binding of GSK 3b to the GSK 3b-binding domains of conductine.